

What is claimed:

1. An isolated nucleic acid molecule comprising:
 - 5 a) a promoter, wherein the activity of the promoter is dependent on the presence of the human immunodeficiency virus (HIV) Tat protein;
 - b) at least one splice donor site and at least one splice acceptor site;
 - c) an expressible sequence which is not a wild-type HIV sequence, wherein at least part of the expressible sequence is located in an intron
 - 10 d) a Rev Responsive Element (RRE) from the human immunodeficiency virus,wherein elements (a)-(d) are operably linked; or a complement thereof.
- 15 2. The nucleic acid molecule of claim 1; wherein the promoter comprises a human HIV 5' long terminal repeat (LTR) or a portion thereof; or a complement thereof.
3. The nucleic acid molecule of any one of claims 1 or 2, further comprising a human HIV 3' LTR; or a complement thereof.
- 20 4. The nucleic acid molecule of any one of claims 1-3, wherein the splice donor site is the HIV D1 splice donor site; or a complement thereof.
5. The nucleic acid molecule of any one of claims 1-4, wherein the splice
- 25 acceptor site is the HIV A7 splice acceptor site; or a complement thereof.
6. The nucleic acid molecule of any one of claims 1-5, wherein the splice acceptor site is contained within the RRE; or a complement thereof.
- 30 7. The nucleic acid molecule of any one of claims 1-6, further comprising at least a second splice donor site and at least a second splice acceptor site; or a complement thereof.
8. The nucleic acid molecule of claim 8, wherein the second splice donor site is the HIV D4 splice donor site; or a complement thereof.

9. The nucleic acid molecule of any one of claims 7 or 8, wherein the second splice acceptor site is the HIV A5 splice acceptor site; or a complement thereof.

5 10. The nucleic acid molecule of any one of claims 1-6, wherein the nucleic acid molecule comprises the nucleic acid molecule depicted in Figure 4; or a complement thereof.

11. The nucleic acid molecule of any one of claims 1-9, wherein the nucleic acid molecule comprises the nucleic acid molecule depicted in Figure 5; or a complement thereof.

10 12. The nucleic acid molecule of any one of claims 1-11, further comprising a psi (ψ) site; or a complement thereof.

13. The nucleic acid molecule of any one of claims 1-12, wherein the expressible
15 sequence is a reporter gene; or a complement thereof.

14. The nucleic acid molecule of claim 13, wherein the reporter gene encodes a protein selected from the group consisting of: a fluorescent protein, luciferase, β -galactosidase, chloramphenicol acetyl transferase (CAT), thymidine kinase (TK); or a
20 complement thereof.

15. The nucleic acid molecule of claim 14, wherein the fluorescent protein is selected from the group consisting of green fluorescent protein (GFP), enhanced green fluorescent protein (EGFP), red fluorescent protein (RFP), yellow fluorescent protein (YFP),
25 enhanced yellow fluorescent protein (EYFP), blue fluorescent protein (BFP), and cyan fluorescent protein (CFP); or a complement thereof.

16. The nucleic acid molecule of claim 15, wherein the luciferase is selected from the group consisting of firefly luciferase and *Renilla* luciferase; or a complement thereof.

30 17. The nucleic acid molecule of any one of claims 1-10, wherein the expressible sequence comprises a therapeutic gene; or a complement thereof.

18. The nucleic acid molecule of claim 17, wherein the therapeutic gene encodes a cytotoxic protein; or a complement thereof.

19. The nucleic acid molecule of any one of claims 1-18, further comprising an
5 internal ribosome entry site (IRES); or a complement thereof.

20. The nucleic acid molecule comprising the insert contained within the plasmid deposited with the NIAID Research and Reference Reagent Program as Accession No. ____

10 21. The nucleic acid molecule comprising the insert contained within the plasmid deposited with the American Type Culture Collection as Accession No. _____ .

22. An isolated nucleic acid molecule comprising a nucleic acid sequence selected from the group consisting of: SEQ ID NO:1, SEQ ID NO:2, and SEQ ID NO:3; or a
15 complement thereof.

23. An isolated nucleic acid molecule comprising a nucleic acid sequence which is at least about 60% identical to a nucleic acid sequence selected from the group consisting of: SEQ ID NO:1, SEQ ID NO:2, and SEQ ID NO:3; or a complement thereof.
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24. The nucleic acid molecule of any one of claims 1-23, which is contained within a vector.

25. The nucleic acid molecule of claim 24, wherein the vector is a plasmid.
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26. The nucleic acid molecule of claim 24, wherein the vector is a recombinant virus.

27. The nucleic acid molecule of claim 26, wherein the vector is a recombinant
30 retrovirus.

28. The nucleic acid molecule of claim 27, wherein the vector is a recombinant lentivirus.

29. The nucleic acid molecule of any one of claims 27-28, wherein the retrovirus is derived from HIV.

30. The nucleic acid molecule of any one of claims 26-29, wherein the virus is
5 replication incompetent.

31. A host cell containing the nucleic acid molecule of any one of claims 1-30.

32. The host cell of claim 31, wherein the nucleic acid molecule is stably
10 integrated into the genome of the cell.

33. The host cell of any one of claims 31 or 32, which is a human T cell.

34. The host cell of any claim 33, which is a CEM T cell.
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35. The host cell deposited with the NIAID Research and Reference Reagent Program as Accession No. _____.

36. The host cell deposited with the American Type Culture Collection as
20 Accession No. _____.

37. A method of determining whether HIV is present in a sample comprising:
a) contacting the host cell of any one of claims 31-36 with the
sample;
25 b) culturing the cell for an amount of time sufficient to allow HIV infection and gene expression; and
c) determining whether the reporter gene is expressed by the cell;

wherein expression of the expressible sequence is indicative of the presence of HIV in the sample.

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38. The method of claim 37, wherein the sample is a biological sample isolated from a subject.

39. The method of claim 38, wherein the subject is a human.

40. The method of claim 38, wherein the sample is selected from the group consisting of a biological fluid sample, a tissue sample, and a cell sample.

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41. The method of claim 40, wherein the biological fluid is selected from the group consisting of blood, serum, plasma, saliva, urine, stool, semen, vaginal fluid, spinal fluid, lymph, amniotic fluid, tears, nasal secretions, sweat, breast milk, mucus, and interstitial fluid.

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42. The method of claim 40, wherein the tissue sample is selected from the group consisting of a lymph node sample, a skin sample, and a chorionic villus sample.

43. The method of claim 40, wherein the cell sample is a blood cell sample.

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44. The method of claim 43, wherein the cell sample is a T cell sample.

45. The method of any one of claims 37-44, wherein the sample is purified.

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46. A method of determining whether a cell is infected with HIV comprising:

- a) contacting the cell with the virus of any one of claims 26-30;
- b) culturing the cell for an amount of time sufficient to allow HIV gene expression; and

25 c) determining whether the expressible sequence is expressed by the cell;
wherein expression of the expressible sequence is indicative of HIV infection of the cell.

47. The method of claim 46, wherein the cell is a T cell.

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48. A method of determining whether a subject is infected with HIV comprising:

- a) contacting the cells of the subject with the virus of any one of claims 26-30; and
- b) determining whether the expressible sequence is expressed by the cells;

wherein expression of the expressible sequence is indicative of HIV infection.

49. The method of claim 48, wherein the subject is a human.

5 50. The method of any one of claims 37-49, wherein the step of determining whether the expressible sequence is expressed by the cell(s) comprises detecting the RNA encoded by the expressible sequence.

10 51. The method of claim 50, wherein the RNA is detected using a method selected from the group consisting of Northern blotting, primer extension, RT-PCR, and nuclease protection.

15 52. The method of any one of claims 37-49, wherein the step of determining whether the expressible sequence is expressed by the cells comprises detecting the polypeptide encoded by the expressible sequence.

53. The method of claim 52, wherein the polypeptide is detected using a method selected from the group consisting of Western blotting, ELISA, and RIA.

20 54. The method of claim 52, wherein the polypeptide is detected using a method that detects the activity of the polypeptide.

25 55. The method of claim 54, wherein the method that detects the activity of the polypeptide is selected from the group consisting of a fluorescence assay, a β -galactosidase assay, a CAT assay, a luciferase assay, and a thymidine kinase assay.

30 56. A method of killing a cell infected with HIV comprising contacting the cell with the virus of any one of claims 29-30, wherein the expressible sequence encodes a cytotoxic protein.

57. The method of claim 56, wherein the cell is a T cell.

58. The method of any one of claims 56-57, wherein the cells are contained within a human subject.

59. A method of treating a subject infected with HIV comprising administering to
5 the subject the virus of any one of claims 26-30, wherein the expressible sequence encodes a cytotoxic protein.

60. A method of identifying a compound capable of inhibiting HIV infection or gene expression or comprising:
10 a) contacting the host cell of any one of claims 31-36 with a test compound;
b) contacting the cell with HIV;
c) culturing the cell for an amount of time sufficient to allow HIV infection and gene expression; and
15 d) determining whether the expressible sequence is expressed by the cell,
wherein a compound that inhibits expression of the expressible sequence is identified as a compound that is capable of inhibiting HIV infection or gene expression.

61. The method of claim 60, wherein steps (a) and (b) may be performed in any
20 order or at the same time.

62. A method of identifying a compound capable of inhibiting HIV infection or gene expression or comprising:
25 a) contacting a cell with HIV;
b) contacting the cell with the virus of any one of claims 26-30;
c) contacting the cell with a test compound;
d) culturing the cell for an amount of time sufficient to allow HIV infection and gene expression; and
e) determining whether the expressible sequence is expressed by the cell,
30 wherein a compound that inhibits expression of the expressible sequence is identified as a compound that is capable of inhibiting HIV infection or gene expression.

63. The method of claim 62, wherein steps (a), (b), and (c) may be performed in any order or at the same time.

64. A method of identifying a compound capable of inhibiting HIV infection or
5 gene expression or comprising:
a) contacting the cell infected with HIV with the retrovirus of any one of
claims 26-30;
b) contacting the cell with a test compound;
c) culturing the cell for an amount of time sufficient to allow HIV
10 infection and gene expression; and
d) determining whether the expressible sequence is expressed by the cell,

wherein a compound that inhibits expression of the expressible sequence is identified as a compound that is capable of inhibiting HIV infection or gene expression.

15 65. The method of claim 64, wherein steps (a) and (b) may be performed in any order or at the same time.

66. The method of any one of claims 60-65, wherein the step of determining whether the expressible sequence is expressed by the cell comprises detecting the RNA
20 encoded by the reporter gene.

67. The method of claim 66, wherein the mRNA is detected using a method selected from the group consisting of Northern blotting, primer extension, RT-PCR, and nuclease protection.
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68. The method of any one of claims 60-65, wherein the step of determining whether the expressible sequence is expressed by the cells comprises detecting the polypeptide encoded by the reporter gene.

30 69. The method of claim 68, wherein the polypeptide is detected using a method selected from the group consisting of Western blotting, ELISA, and RIA.

70. The method of claim 68, wherein the polypeptide is detected using a method that detects the activity of the polypeptide.

71. The method of claim 70, wherein the method that detects the activity of the
5 polypeptide is selected from the group consisting of a fluorescence assay, a β -galactosidase assay, a CAT assay, a luciferase assay, and a thymidine kinase assay.